EFFECTS OF SECRETIONS OF CERTAIN PARASITIC NEMATODES ON COAGULATION OF BLOOD.[1]

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INTRODUCTION

The fact that certain parasitic nematodes, especially some members of the family Strongylidae, a group that includes the hookworms, have the power of lacerating the intestinal mucosa, places these parasites in the category of serious pathogenic agents. In considering the question of their pathogenicity, in addition to the damage done by the abstraction of blood and by the mechanical injury to the mucosa of the intestine, caused by their bites, including the entrance of bacteria, it is important to consider the possible effects of the secretions of the worms on the intact as well as on the injured intestinal mucosa and the general effects on the host of the absorption of these secretions into the circulation. Among the toxic products elaborated by these nematodes substances that retard coagulation of blood have been found in certain species. That these substances are responsible for the persistent oozing of blood from wounds inflicted by hookworms and related nematodes, appears probable.

A record and discussion of the writer's experiments on effects of extracts of certain nematodes on coagulation of blood is given in the following pages. No attempt has been made to correlate the results of these experiments with theories of blood coagulation.

REVIEW OF LITERATURE

That the secretions of certain nematodes have the power of retarding coagulation of blood *in vitro* was first shown by Loeb and Smith in 1904. These investigators found that extracts of *Ancylostoma caninum* in physiological salt solution inhibit the coagulation of dogs' blood *in vitro* for periods which vary with different samples of blood, the maximum period of delay in coagulation observed by these writers being about twenty-four hours. Loeb and Smith found, moreover, that the substance involved in this process is present in the anterior half of the worms and is completely absent in the posterior half. The substance was found by these writers to be highly resistant to heat since

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fifteen minutes' boiling merely weakened but did not destroy its power to inhibit coagulation of blood.

Although the work and conclusions of Loeb and Smith are questioned by Liefmann (1905), experimental work by Loeb (1906) and Loeb and Fleisher (1910) showed quite conclusively the presence in the anterior portion of the body of *Ancylostoma caninum* of a substance that retards coagulation of dogs' blood, and confirmed the conclusions of the earlier work of Loeb and Smith.

Weinberg (1907) in the course of his investigations on effects of extracts of horse strongyles, belonging to the genus Strongylus, on the blood of the horse, found that physiological salt solution extracts of triturated specimens of these worms inhibited coagulation of horses' blood, since mixtures of the freshly drawn blood and extracts in question were still uncoagulated after four days.

Aside from the experimental work with extracts of worms belonging to the genera Ancylostoma and Strongylus, there appears to exist some evidence in favor of the view that the fluid which occurs in the body cavity of worms belonging to the genus Ascaris has the power of inhibiting to a certain extent coagulation of blood *in vitro*. Leroy (1910), experimenting with dogs, and Weil and Boyé (1910), experimenting with rabbits, found that the blood of animals which had been injected with the body fluid of ascarids coagulated more slowly than the blood of non-injected animals, but Weil and Boyé failed to observe that the fluid had any effect on the coagulation of rabbits' blood *in vitro*. Flury (1912), on the other hand, found that the fluid delayed coagulation of dogs' blood and human blood *in vitro*.

EXPERIMENTS BY THE WRITER

Experiments by the writer have been made with physiological salt solution extracts of *Strongylus vulgaris, Strongylus edentatus, Bustomum phlebotomum, Bustomum trigonocephalum, Stephanurus dentatus, Oesophagostomum columbianum, Dictyocaulus filaria, Haemonchus contortus, Ascaris lumbricoides, Ascaris equorum,* and *Belascaris* sp. The extracts in question were prepared from specimens collected shortly after the death of the host. The specimens were washed in physiological salt solution, dried between layers of filter paper and exposed to room temperature or to a temperature of 37° C. until they became sufficiently crisp to be pulverized. A quantity of powder was then added to physiological salt solution in a test tube, the contents were thoroughly shaken and allowed to remain in a refrigerator at a temperature of about 10° C. for about twenty-four hours. Before being used in experiments, extracts prepared as outlined above were filtered through ordinary filter paper. In nearly all experiments referred to in the following pages about 0.1 gm. of powder of the

dried parasite in question was added to each cubic centimeter of physiological salt solution. Equal parts of freshly drawn blood and of extract were used in each experiment. Each experiment was controlled by adding to a quantity of the blood that was used in the test an equal volume of physiological salt solution.

Weinberg's conclusions concerning the presence in worms belonging to the genus Strongylus of a substance that inhibits coagulation of blood were confirmed. Extracts of *Strongylus edentatus* and of *Strongylus vulgaris* inhibited coagulation of rabbits' blood for periods ranging from thirty minutes to sixty minutes, as compared with controls. Rabbit blood in contact with extracts of specimens of *Strongylus edentatus* that had been preserved in alcohol for several weeks showed no delay in coagulation. The substance in these worms that delays coagulation of blood is evidently less potent for rabbit blood than for horse blood, which probably indicates that it has a selective action on the blood of its host. That this substance is not limited to the anterior part of the worm, as is the case in worms of the genus Ancylostoma, was shown by the following experiment:

The anterior portions (roughly about one-third of the total length of the worms) of seven dried specimens of *Strongylus edentatus* were triturated in a mortar and extracted in one cubic centimeter of physiological salt solution. The remaining posterior portions of these specimens were also triturated and extracted in an equal quantity of salt solution. Freshly drawn rabbit blood in contact with the above extracts remained uncoagulated one hour, whereas the control was coagulated in five minutes. The blood in the tube containing the extract of the posterior portion was still fluid when that in the tube containing the extract of the anterior portion was beginning to coagulate, but the difference between the rapidity of coagulation of the two samples of blood was only five minutes.

A series of experiments was performed with extracts of cattle hookworms *(Bustomum phlebotomum)*. Experiments 1 to 5 were performed with five different samples of freshly drawn cattle blood.

Experiment 1: The blood remained uncoagulated for thirty minutes. The blood in the control tube coagulated in ten minutes.

Experiment 2: The blood remained uncoagulated two and one-half hours. The blood in the control tube became coagulated in ten minutes.

Experiment 3: The blood remained uncoagulated for two and one-half hours. The blood in the control tube became coagulated in fifteen minutes.

Experiment 4: The blood remained uncoagulated three and one-half hours. The blood in the control tube became coagulated in fifteen minutes.

Experiment 5: The blood was still uncoagulated after twenty-four hours. The blood in the control tube became coagulated in ten minutes.

Experiment 6: Rabbit blood was used in this experiment. The blood remained uncoagulated for fifty minutes. The blood in the control tube was coagulated in seven minutes.

In the series of experiments upon cattle blood it was observed that only a portion of the blood actually coagulated. In the control tubes the blood clot was from two to three times as large as that in the tubes containing the extract. The latter showed a heavy sediment of erythrocytes, whereas the control tubes showed but a slight sediment of red blood corpuscles.

Experiments with extracts of a closely related species, namely, *Bustomum trigonocephalum,* a hookworm parasitic in sheep, yielded the following results: Two samples of rabbits' blood showed a delay in coagulation of twenty minutes as compared with the controls, and one sample of cattle blood showed a delay of forty-five minutes as compared with the control.

In order to determine whether the substance in the worms that inhibits coagulation of blood is readily soluble in salt solution, powder used in experiments 1 to 6 which had been extracted once was re-extracted and tested on samples of cattle blood with the following results: In two cases no effects were produced, since coagulation occurred in the controls and in the tests at the same time. In one case coagulation was delayed ten minutes as compared with the control and in another case it was delayed fifteen minutes, thus showing that the first extraction removed practically the entire anticoagulin from the parasite material.

Extracts of the stomach worm *(Haemonchus contortus)* were tested on ten samples of sheep blood, on five samples of cattle blood, and on several samples of rabbit blood. In nearly all cases the blood in contact with the extracts coagulated more slowly than the controls, but the maximum delay in coagulation of blood in contact with *Haemonchus contortus* extract as compared with the controls was about fifteen minutes.

Extracts of the kidney worm of swine *(Stephanurus dentatus),* of the lungworm of sheep *(Dictyocaulus filaria),* and of the gapeworm of poultry *(Syngamus trachealis)* were tested on three samples of sheep blood with negative results.

Extracts of *Oesophagostomum columbianum,* the nodular worm of sheep, prepared by macerating twelve fresh specimens in 2 c.c. of physiological salt solution, produced no effect on two samples of rabbit blood and one sample of cattle blood.

The negative results obtained in these experiments, as well as the weakly positive results obtained with *Haemonchus contortus,* serve as a control on the specificity of the reaction with extracts of species of Ancylostoma, Bustomum, and Strongylus, and show quite conclusively that the substance or substances in the worms which inhibit coagulation of blood are specific anticoagulins physiologically related to hirudin and certain snake venoms, and not merely mixtures of

proteins in solution. While solutions of proteoses, of trypsin, of pepsin, and extracts of tissues are known to retard coagulation of blood when injected into the living animal, they have been shown not to delay coagulation of blood *in vitro,* and hence the effects on coagulation of blood *in vitro* produced by extracts of certain nematodes cannot be ascribed to such substances.

A series of experiments with *Ascaris lumbricoides* fluid and rabbit blood yielded the following results: Three to five drops of fluid of *Ascaris lumbricoides* from swine in contact with ten drops of blood produced a delay in coagulation of fifteen minutes as compared with the control. A mixture of eight drops of fluid and ten drops of blood remained uncoagulated thirty-five minutes longer than the control. A mixture of ten drops of fluid and ten drops of blood showed a delayed coagulation of forty-two minutes as compared with the control.

Extracts of *Ascaris equorum* and of *Belascaris* sp. were tested on three samples of sheep blood with weakly positive results, i. e., blood in contact with these extracts remained uncoagulated five to fifteen minutes longer than the controls.

These experiments confirm Flury's results with human blood and dog blood and show that the fluid that is present in the body cavity of *Ascaris lumbricoides* delays to a certain extent coagulation of blood *in vitro.*

DISCUSSION

That certain nematodes secrete substances that have toxic properties and that are absorbed by the host is a view which has been advanced by a number of investigators. In addition to the toxic principle discussed in this paper, other specific toxic substances, especially hemolysins, have been shown to occur in several species. It is probable that the toxic secretions of nematodes, like snake venoms, are a complex of a number of different principles, such as hemolysins, anticoagulins, and one or more systemic poisons.

It is of interest to note that nematodes that contain anticoagulins also contain hemolysins.[3] The former are probably distinct from the latter chemically as well as physiologically. The hemolysin found in species of Ancylostoma is destroyed by heating at 55° C. for several minutes (Whipple, 1909; Schwartz, 1920), whereas the anti-coagulin in these worms resists boiling (Loeb and Smith, 1904). Further studies on the properties of anticoagulins from nematodes would probably yield interesting comparisons with those of hemolysins. Investigations concerning the possible presence of hemolysins in nematodes that lack anticoagulins would also be of interest.

3. A brief account of hemolysins from parasitic worms is given in a recent paper by the writer (Schwartz, 1920).

It is conceivable and by no means improbable that substances in nematodes which delay coagulation of blood may be of etiological significance in the pathology of nematode diseases. Loeb and his collaborators are inclined to the view that the oozing of blood from the wounds inflicted by hookworms, rather than the absorption by the host of a hemolysin elaborated by the parasites, accounts for the anemia that occurs in cases of infestation with these parasites. That the anticoagulin which has been shown to occur in the anterior portion of these parasites is responsible for the persistent hemorrhages in question appears to be a warranted conclusion. In this connection it is of interest to observe that markedly strong anticoagulins, so far as is known, occur only in nematodes belonging to the family Strongylidae, the members of which commonly produce pronounced anemia in the host.

SUMMARY

1. The substance in species of Strongylus that inhibits coagulation of blood is present in the posterior as well as in the anterior portion of the worms.

2. *Bustomum phlebotomum,* a hookworm parasitic in cattle, contains a substance soluble in salt solution that inhibits coagulation of blood for considerable periods which vary with different samples of blood. A closely related species, *Bustomum trigonocephalum,* contains a similar anticoagulin.

3. Salt solution extracts of *Haemonchus contortus* cause but a slight delay in coagulation of blood. Extracts of *Syngamus trachealis, Dictyocaulus filaria,* and *Stephanurus dentatus* do not retard coagulation of sheep blood. Extracts of *Oesophagostomum columbianum* do not retard coagulation of rabbits' blood.

4. The body fluid of *Ascaris lumbricoides* inhibits coagulation of blood to a moderate extent. Extracts of *Ascaris equorum* and of *Belascaris* sp. have a slight effect on coagulation of sheep's blood.

5. In view of the fact that the delay in coagulation of blood due to extracts of nematodes occurs *in vitro,* that it varies with extracts of different species of worms, and that extracts of certain species produce no delay in coagulation, it may be concluded that specific substances, other than proteins in solution, must be involved.

6. The substances in question appear to be physiologically related to hirudin and snake venom, and like the latter are probably part of a complex of toxic principles.

7. So far as present knowledge goes, nematodes which contain substances that inhibit coagulation of blood to a marked degree are zoologically related, belonging to the family Strongylidae, the mem-

bers of which have a buccal capsule adapted to lacerating the intestinal mucosa.

8. That the injection of their secretions into the intestinal mucosa, by certain biting nematodes, resulting in minute hemorrhages, is of etiological importance in nematode diseases appears very probable.

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